

## Diagnostic utility of haematological parameters and C-reactive protein in early detection of neonatal septicaemia in developing countries

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### Abstract

**Introduction:** Septicaemia is one of the major causes of mortality and morbidity during the neonatal period especially in developing countries. Early diagnosis of neonatal sepsis is a diagnostic problem due to non-specific clinical features. Blood culture is the gold standard, but it takes several days and is expensive. Hence there is a critical need for laboratory tests that aid in the rapid diagnosis of neonatal sepsis.

**Aims and Objectives:** To measure the role of Haematological Scoring System and C-reactive protein as an early diagnostic tool for neonatal sepsis.

**Materials and Methods:** This was a prospective study performed on 116 neonates, who were clinically suspected cases of septicaemia, admitted at Neonatal Intensive Care Unit, Yenepoya Hospital, Mangalore. Each hematologic parameter like total leukocyte count, polymorphonuclear neutrophil (PMN) count, immature PMN count, platelet count etc. were analysed in all cases using the Haematological Scoring System (HSS) formulated by Rodwell et al. Blood cultures were performed as a gold standard. C-reactive protein (CRP) was also estimated.

**Results:** Out of 116 neonates, 61(52%) were male neonates and 55(48%) were female neonates. 18(15%) cases were with proven sepsis, 58(50%) cases with probable sepsis and 40(35%) cases were on the safer side. The HSS was found to have a sensitivity of 94.4%, specificity of 75.5%, positive predictive value was 41.4% and negative predictive value was 98.6%.

**Conclusions:** HSS is a rapid, simple and easily affordable screening tool for early detection of neonatal septicaemia. It also provides an effective guideline to make decisions regarding judicious use of antibiotics.

**Keywords:** Blood culture, Haematological Scoring System, Neonates, Septicaemia, Rapid diagnosis.

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### Introduction

Neonatal septicaemia is a systemic infection seen in the infants before 28 days of life.<sup>1</sup> Nearly one million deaths per year are caused by neonatal sepsis, accounting for over 25% of global neonatal deaths. Majority of these deaths occur in developing countries.<sup>2</sup> As per National Neonatal Perinatal Database (NNPD) 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live birth.<sup>3</sup>

The protection provided by effective barriers like placenta and amniotic sac gets exposed to the microbial world during birth.<sup>4</sup> The most common organism is *Klebsiella pneumonia* followed by *Staphylococcus aureus* and *Pseudomonas* in India.<sup>5</sup> Isolation of the causative microorganisms by using blood culture is the gold standard method for diagnosis, but it takes 24-72 hrs for obtaining the reports and in some cases possibility of false negative result may be present.<sup>6,7</sup> Recently various markers which are both sensitive and specific have been evaluated but they are expensive and sophisticated.<sup>8</sup> Hence rapid and cost effective methods like Haematological Scoring System (HSS) that

includes white blood cell count, absolute neutrophil count, platelet count and C-reactive protein(CRP) estimation have been evaluated in this study to know its diagnostic role in developing countries.<sup>9</sup>

### Materials and Methods

This was a prospective study performed on 116 neonates, who were clinically suspected cases of septicaemia or with predisposing perinatal factors, admitted at Neonatal Intensive Care Unit of our institution. Detailed history was taken and the information was recorded in a specifically designed proforma. The neonates were divided into 3 groups. (Table 1)

The collection of blood samples were done by peripheral venepuncture in an ethylene diamine tetra acetic acid (EDTA) vial. The total WBC counts were counted on a Sysmex counter (Sysmex XS 1000i) and corrected for nucleated red blood cells. Differential counts were performed on blood smears stained by Leishman stain, counting 100 cells. All smears were reviewed by a consultant pathologist blinded to the infection status of the infants. The

haematological findings were analyzed according to the Haematological Scoring System (HSS) of Rodwell et al.<sup>10</sup> The HSS assigns a score of one for each of the seven criteria found to be significantly associated with sepsis like total WBC count, total polymorphonuclear (PMN) leucocyte count, Immature:total (I:T) PMN count etc (Table 2) with one exception. An abnormal total PMN count is assigned a score of 2 instead of 1, if no mature are seen on the peripheral smear to compensate for the low immature:mature (I:M) PMN ratio. Immature neutrophils include promyelocyte, myelocyte, metamyelocytes, and band form. (Fig.1a, b) Degenerative changes in neutrophils include vacuolization (Fig. 1c), toxic granulations, and Dohle bodies. Interpretation is shown in Table 3. Minimum score is 0 and maximum score is 8. Blood cultures were performed as a gold

standard. CRP was also estimated. Nucleated red blood cells (NRBC) were counted in each case. Sensitivity, specificity, and positive and negative predictive values (PPVs and NPVs) were evaluated for each of the individual criteria and for the overall score of HSS and for CRP using standard statistical methods. P value <0.05 was considered as statistically significant.<sup>14</sup>

**Inclusion Criteria:** All clinically suspected cases of neonatal sepsis were included in the study.

**Exclusion Criteria:** Neonates of mothers with diabetes, pregnancy induced hypertension and neonates with history of asphyxia and congenital anomalies, prematurity (<30 weeks), blood group incompatibility, any intake of previous antibiotic were excluded by a detailed perinatal history and clinical examination.

**Table 1: Categorization of study population.**

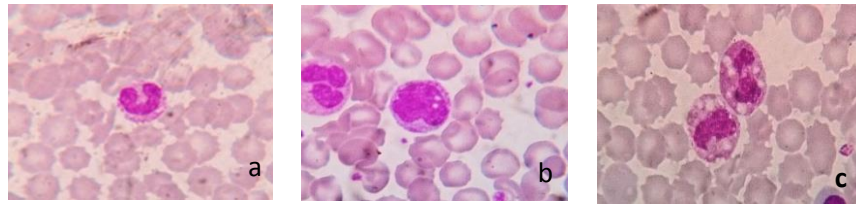
| Groups | Presentation  |
|--------|---|
| 1      | Sepsis with positive blood culture                                      |
| 2      | Probable sepsis with strong clinical history but negative blood culture |
| 3      | Normal infants with minimal evidence of sepsis                          |

**Table 2: Haematological scoring system**

| Criteria                    | Abnormality                          | Score |
|-----------------------------|--------------------------------------|-------|
| Total WBC Count             | ≤5000/cumm                           | 1     |
|                             | ≥25,000 at birth                     |       |
|                             | ≥30,000 at 12-24 hours               |       |
|                             | >21,000—Day 2 onwards                |       |
| Total PMN Count             | No mature PMN                        | 2     |
|                             | Increased (>5,400/cumm)              | 1     |
| Immature PMN Count          | Increased (≥600/cumm)                | 1     |
| I:T PMN Ratio               | Increased (≥0.12)                    | 1     |
| I:M PMN Ratio               | Increased(≥0.3)                      | 1     |
| Degenerative Changes In PMN | Toxic granules/ cytoplasmic vacuoles | 1     |
| Platelet Count              | ≤1,50,000/cumm                       | 1     |

**Table 3: Interpretation of Haematological Scoring System**

| Score  | Interpretation                     |
|--------|------------------------------------|
| ≤2     | Sepsis is very unlikely            |
| 3 or 4 | Probable sepsis                    |
| >5     | Sepsis or infection is very likely |



**Fig.1: Photomicrograph of peripheral smear showing: a) and b): Immature neutrophils; c) Neutrophils with cytoplasmic vacuoles**

## Result

Out of 116 neonates who were clinically suspected of sepsis, 61(52%) were male and 55(48%) were female neonates, with Male: Female ratio of 1.1:1. Maximum number (68; 58.6%) of patients belonged to the age group 1-13 days. Maximum cases (42%) belonged to low birth weight group of 1 to <2.5 kg. 18(15%) cases were with proven sepsis, 58(50%) cases with probable sepsis and 40(35%) cases were on the safer side. *Escherichia coli* was the most common organism isolated by blood culture followed by *Staphylococcus aureus* and *Enterobacter spp.*

Table 4 shows scores of each group. 17 (94.4%) neonates with proven sepsis had scores  $\geq 5$ . 1(5.6%) neonates with proven sepsis had scores 3-4. 21 (36.2%) neonates with history of probable infection had scores  $\geq 5$  and 29(50%) had scores 3-4 suggesting the possibility of sepsis. 3 (7.5%) of normal neonates had score  $\geq 5$  and 7 (17.5%) had scores 3 - 4 suggesting

the possibility of sepsis in these cases. 20 (50%) of normal neonates and 8 (13.7%) neonates with probable infection had score  $\leq 2$ , which implies sepsis was unlikely in these cases.

In this study, (Table 5) individual parameters like I:T PMN ratio and Immature PMN counts showed high sensitivity (100%). Total WBC count (83.6%) was highly specific followed by platelet count (78.5%) in identifying neonates with sepsis. Negative predictive value was high in I:T PMN ratio and Immature PMN count (100%) followed by HSS(98.6%) which was useful in identifying neonates that did not have any evidence of sepsis. The HSS was found to have a sensitivity of (94.4%), specificity of (75.5%), positive predictive value was (41.4%) and negative predictive value was (98.6%). CRP provides limited sensitivity (44.4%) but provides very high negative predictive values (87.9%) and is thus useful for identifying infants unlikely to be infected. Nucleated Red blood cells values were higher in sepsis and significantly seen in 70% of cases in proven sepsis.

**Table 4: Distribution of cases according to sepsis score**

| Category             | Scores 0-2 | Scores 3-4 | Scores $\geq 5$ |
|----------------------|------------|------------|-----------------|
| Proven sepsis (18)   | 0          | 1          | 17              |
| Probable sepsis (58) | 8          | 29         | 21              |
| No sepsis (40)       | 20         | 17         | 3               |
| <b>Total (116)</b>   | 28         | 47         | 41              |

**Table 5: Performance of individual haematological parameter & CRP**

| Criteria           | Sensitivity (%) | Specificity (%) | Positive Predictive Value (%) | Negative Predictive Value (%) |
|--------------------|-----------------|-----------------|-------------------------------|-------------------------------|
| HSS                | 94.4            | 75.5            | 41.4                          | 98.6                          |
| Total WBC Count    | 38.8            | 83.6            | 30.4                          | 88.1                          |
| Total PMN Count    | 83.3            | 33.6            | 18.7                          | 91.6                          |
| Immature PMN Count | 100             | 24.4            | 19.5                          | 100                           |
| I:T PMN Ratio      | 100             | 27.5            | 20.2                          | 100                           |
| I:M PMN Ratio      | 72.2            | 69.3            | 30.2                          | 93.1                          |
| Platelet Count     | 66.6            | 78.5            | 36.3                          | 92.7                          |
| CRP                | 44.4            | 74.4            | 24.2                          | 87.9                          |

## Discussion

In developing countries like India, neonatal sepsis remains a problematic disease and is potentially life-threatening. Some of the risk factors for sepsis include maternal factors like premature rupture of membranes, maternal infections, procedures etc. and infant factors like low birth weight, poor cord care, congenital anomalies, low APGAR score etc.<sup>1,2,4</sup> Clinically the patients may present with respiratory distress, irritability, hypothermia, hypo or hyperglycaemia, vomiting, poor feeding, cyanotic spells, seizures and shock.<sup>6</sup> It is a great challenge to diagnose neonatal septicaemia as the early signs of sepsis may be subtle and different at different gestational ages.<sup>11</sup> The evaluation of screening tests for neonatal sepsis is an absolute necessity as the infection may present a serious threat to the baby. Non infected neonates should also be identified to avoid unnecessary antibiotic administration and emergence of resistant microorganisms.<sup>12, 14-16</sup>

An ideal screening test should have high sensitivity and high negative predictive value that would detect all the patients with the disease and exclude all the cases, which do not have that specific disease. The risk of missing a patient with a certain infection is greater than the risk of over treatment with antibiotics hence a low specificity and positive predictive value are acceptable.<sup>6</sup> Even though blood culture is gold standard test for diagnosing sepsis, the procedure is expensive, time consuming as it takes 48-72

hours, has low yield due to insufficient sample volumes, intermittent or low-density bacteraemia, or suppression of bacterial growth by earlier (i.e., intrapartum) antibiotic administration.<sup>17-19</sup> Moreover the technique demands a well-equipped laboratory which is not available in most of the community hospitals.<sup>20-22</sup>

In the recent past, various markers which are both sensitive and specific like ELISA methods, prolactins, haptoglobins, interleukins, molecular analysis by polymerase chain reaction (PCR) for bacterial DNA etc. to diagnose neonatal sepsis have been evaluated but they are expensive, sophisticated and unavailable in rural setups.<sup>1,8,13</sup>

A Hematologic Scoring System (HSS) of Rodwell was introduced for early diagnosis of neonatal septicaemia which is easy to perform and applicable to all infants, including those who have received treatment.<sup>9,10</sup> It included haematological parameters and showed that such score could accurately predict the presence or absence of infection and was reliable.

In our study, among the infected newborns, the predominance of male was seen. The factors that regulate the synthesis of alpha globulin are situated on the X chromosome. Males have only one X chromosome; hence are less immunologically protected than the females.<sup>9</sup> This is consistent with other studies.<sup>3-9,14-16</sup>

In this study, individual parameters like I:T PMN ratio and Immature PMN counts showed high sensitivity and negative predictive value which was consistent with many other studies.(Table 6)

**Table 6: Comparison of sensitivity and NPV of individual parameters with other studies**

| Series             | Highest sensitivity and NPV       |
|--------------------|-----------------------------------|
| Nayana NS et al    | I:T ratio                         |
| Supreetha MS et al | I:M ratio                         |
| Narasimha A et al  | Total PMN count                   |
| Majumdar A et al   | I:T ratio, I:M ratio              |
| Meirina F et al    | I:T ratio, total PMN count        |
| Khair KB et al     | I:T ratio, I:M ratio              |
| Present study      | I:T ratio and Immature PMN counts |

The certainty of sepsis increases as the score increases. HSS score of more >3 was considered significant by various studies.<sup>3-9</sup> A score  $\leq 2$  suggested that sepsis was unlikely. In the present study, HSS was significantly ( $P < 0.005$ ) associated with sepsis. A score of  $\geq 5$  was found to have a sensitivity of 94.4%, specificity of 75.5%, positive predictive value was 41.4% and negative predictive value was 98.6%.

HSS can be considered a superior and more reliable screening tool for sepsis than any of the individual haematological parameter due to

higher specificity and positive predictive value. It simplifies the interpretation of haematological profiles.

Nucleated Red blood cells values were higher in sepsis and significantly seen in 70% of cases in proven sepsis. This was consistent with other studies.<sup>5</sup> Studies showed that mean NRBC value of 10 is seen in term and healthy neonates.<sup>23</sup> NRBC count greater than the indicated reference values may be seen in postnatal hypoxia, anaemia, maternal diabetes or acute stress and sometimes in neonatal

septicaemia. In sepsis, cytokines are released which play an important role in stimulating nucleated RBC production independent of hypoxia.<sup>5</sup>

C-reactive protein which is released in sepsis is a non-specific acute phase protein. Elevated CRP levels are highly suggestive of Neonatal sepsis.<sup>3,6-9</sup> CRP measurement can be done in conjunction with other diagnostic tests such as HSS and blood culture to establish or exclude the diagnosis of sepsis in neonates.<sup>13,15</sup> In the present study CRP >10mg/L was the cut of value to suspect neonatal sepsis.<sup>8</sup> Values of sensitivity & specificity of CRP were 44.4% and 74.4% respectively. PPV of CRP was 24.2% and NPV of CRP was 87.9%. CRP provides high negative predictive values and hence plays an important role in identifying infants unlikely to be infected or for monitoring the response to treatment.

### Conclusion

HSS is a rapid, easy to perform, cost effective and easily available screening system for early detection of neonatal septicaemia. It also provides an effective guideline to make decisions regarding judicious use of antibiotics. Combining it with CRP levels gives a good analysis of sepsis. It has a good sensitivity, rather than high specificity and a good negative predictive value and can therefore help in safely withholding antibiotic administration in non-infected infants. Peripheral blood showing elevated nucleated RBCs values are significantly associated with newborn sepsis and could be an interesting marker of neonatal sepsis in absence of hypoxia.

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